
Histomorphometric analysis of odontoblast-like cell numbers and dentine bridge secretory activity following pulp exposure

P. E. Murray¹, A. A. Hafez², A. J. Smith³, L. J. Windsor¹ & C. F. Cox⁴

¹Oral Biology, Indiana University School of Dentistry, 1121 West Michigan Street, Indianapolis, IN 46202, USA; ²University of Southern California, School of Dentistry, Los Angeles, CA 90007, USA; ³Oral Biology, School of Dentistry, University of Birmingham, St Chad's Queensway, Birmingham B4 6NN, UK; and ⁴Department of Restorative Dentistry, UCLA School of Dentistry, Los Angeles, CA, USA

Abstract

Murray PE, Hafez AA, Smith AJ, Windsor LJ, Cox CF. Histomorphometric analysis of odontoblast-like cell numbers and dentine bridge secretory activity following pulp exposure. *International Endodontic Journal*, **36**, 106–116, 2003.

Aim The purpose of this study was to collect quantitative information about the numbers and dentine bridge secretory activity of odontoblast-like cells following dental pulp exposure.

Methodology The numbers and secretory activity of odontoblast-like cells were measured histomorphometrically between 7 days and 2 years in 161 pulp-exposed nonhuman primate teeth. The area of dentine bridges and the dimensions of cavity preparations were measured. The density of odontoblast-like cells and subjacent reorganizing tissue cells were measured beneath dentine bridge formation. The presence of operative dentine debris and tunnel defects in bridges was noted. Pulp inflammation was categorized according to ISO standards. Bacteria were detected using McKay's stain.

Results The area of dentine bridges was mediated by the density and secretory activity of odontoblast-like cells over time. The cell density of subjacent reorganizing tissue was found to be strongly associated with that of odontoblast-like cells. Bacterial microleakage was found to impede dentine bridge secretion by odontoblast-like cells.

Conclusions Pulp reparative activity occurs naturally beneath capping materials in the absence of bacterial microleakage. The outcome of pulp-capping treatments could be beneficially influenced by concentrating attention on limiting the width of pulp exposure, minimizing pulp injury by limiting the creation of operative debris and placing materials which prevent bacterial microleakage.

Keywords: bacterial leakage, cavity preparation, inflammation, injury, regeneration.

Received 12 March 2002; accepted 16 October 2002

Introduction

The dental pulp has a well-documented ability to form hard-tissue barriers called dentine bridges following direct pulp capping or pulpotomy (Schröder 1985, Tziafas 1994). Following pulp exposure, primary odontoblasts are often irreversibly injured. Odontoblasts are postmitotic terminally differentiated cells, and cannot

proliferate to replace subjacent irreversibly injured odontoblasts. Consequently, the origin of odontoblast-like cells, which secrete dentine bridges following pulp exposure, has proved to be controversial. Autoradiographic studies have indicated that new odontoblast-like cells may be derived from other pulp cell populations by a process of differentiation (Fitzgerald *et al.* 1990). However, the mechanisms by which pulp cells differentiate into odontoblast-like cells and secrete dentine bridges in the absence of basement membrane or dental epithelium are not fully understood (Tziafas 1994).

The replacement of irreversibly injured odontoblasts by predetermined odontoblast-like cells that do not

Correspondence: Dr Peter E. Murray, Oral Biology, Indiana University School of Dentistry, 1121 West Michigan Street, Indianapolis, IN 46202-5186, USA (Tel.: +1 317 274 2126; fax: +1 317 278 1411; e-mail: petmurra@iupui.edu).

replicate their DNA after induction has been suggested. Initially, the progenitors for odontoblast-like cells were thought to be located within the cell-rich subodontoblast layer. However, a study by Fitzgerald *et al.* (1990) showed that at least two replications of DNA are required to have functional odontoblast-like cells at the site of pulp exposure. The first replication takes place before cell migration, and the second at the site of expression of the new odontoblast-like phenotype. This cell migration suggested that the progenitor cells are derived from other cell populations located within the pulp core. The most likely progenitor cell population was assumed to be the fibroblasts located in the pulp core (Fitzgerald *et al.* 1990). Whilst cultured fibroblast cell lines have the ability to migrate in response to cytokine signalling molecules, cultures of pulp fibroblasts did not appear to differentiate into cells with odontoblast-like phenotypes or secretory characteristics (Hanks *et al.* 1998). Nevertheless, human dentine production can be observed from explanted tooth pulp tissue *in vitro* (About *et al.* 2000), indicating that new odontoblast-like cells may originate from other pulp cell populations. Feit *et al.* (1970) demonstrated that undifferentiated progenitor cells of the pulp parenchyma divide and migrate toward the site of pulp exposure, where dentine bridges are secreted. This cellular differentiation pattern provided some support for the theory that the odontoblast-like progenitor cells were resident undifferentiated mesenchymal cells (D'Souza *et al.* 1995). However, recent attention has focused on cells associated with pulp vasculature, most probably pericytes (Carlisle *et al.* 2000) or pericyte progenitor cells such as myofibroblast cells (Alliot-Licht *et al.* 2001). Pericytes are solitary vascular smooth muscle cells associated with the finest diameter blood vessels. It is commonly accepted that pericytes represent pluripotent progenitor cells in the adult and their migration and differentiation have been reported to occur during angiogenesis and tissue repair. In the human dental pulp, pericyte migration and differentiation have been observed (Carlisle *et al.* 2000). However, the transition of these cells to a fibroblast phenotype may explain the difficulty in distinguishing these cells from the endogenous fibroblasts of the pulp core.

The aim of this study was to investigate the kinetics of odontoblast-like cell formation and dentine bridge secretory activity in 161 pulp-capped nonhuman primate teeth. In addition, because information is lacking about the effects and importance of many pulp-capping variables on the kinetics of odontoblast-like cell and dentine bridge formation, this study also assessed the effects of the pulp-capping material, operative debris (OD) com-

posed of dentine chip fragments, as well as bacterial microleakage, tunnel defects in dentine bridges, pulp inflammation, and dimensions of cavity preparations and pulp exposure. These factors may explain how post-operative complications with dentine bridge formation develop at the cellular level.

Materials and methods

Cavity preparation

Ten rhesus monkeys (*Macaca mulatta*), approximately 4–5 years old, were used to study a total of 161 vital teeth. The standardized methods and procedures in this study have been described more completely elsewhere (Cox *et al.* 1987, Tarim *et al.* 1998). Briefly, Class V cavity preparations were placed in teeth evenly distributed amongst the monkey dentition, to minimize any possible differences between animals. Pulp exposure was achieved with a size 330 carbide bur at ultra-high speed under sterile water spray coolant. Haemorrhage was controlled with a cotton pellet dampened with 5.25% NaOCl solution, being held in place for 20–50 s; the cavity was rinsed with sterile saline and gently air dispersed.

Pulp capping

Cavities were immediately direct pulp capped with calcium hydroxide (Ca(OH)₂), composite resin (CR) or resin-modified glass ionomer (RMGI) according to the manufacturers' instructions. The treatment groups were evenly distributed amongst animals. Seventy-six exposed pulps were direct pulp capped with Ca(OH)₂ (Life, Kerr Co., Orange County, CA, USA; Dycal, Caulk Dentsply, Milford, DE, USA). All Ca(OH)₂ cavities were sealed to the cavosurface margin with zinc oxide-eugenol. Fifty-nine pulps were direct pulp capped with CR (All bond + Elitefil, Bisco Co., Schaumburg, IL, USA; Liner Bond II + Clearfil AP-X, Kuraray Co., Osaka, Japan), and 36 exposures capped with RMGI (Vitremer, 3M Dental, St Paul, MN, USA; Dual-cure One Step, Bisco Co., Schaumburg, IL, USA).

Histological observations

Teeth were collected following ISO usage guidelines (Technical Report 7405) at 7, 27, 97 days and 1 and 2 years. Tooth specimens were fixed, demineralized, processed and evaluated according to ISO usage guidelines. Serial 7-µm sections were cut, mounted on glass slides and stained with haematoxylin and eosin or McKay's for identification of bacteria.

Histological analysis

Histological tooth specimens were examined under light microscopy. The inflammatory response of each pulp was categorized in increasing order of severity from 'none', 'slight', and 'moderate' to 'severe' pulp (Table 1), according to Federation Dentaire Internationale and International Organization for Standardization standards and published criteria (Mjör 1983). Bacterial contamination of each restoration was assessed using

McKay's stain (McKay 1970) to detect for presence of gram-positive and -negative microorganisms. The dentine bridge was identified as the formation of tertiary dentine by newly differentiated odontoblast-like cells, at the site of pulp exposure (Table 1). The presence of operative debris (Table 1), including dentine chips, particles of capping material and globules of adhesive (Murray *et al.* 2002), in addition to tunnel defects (Table 1) including tissue tracts (Cox *et al.* 1996), were observed at $\times 100$ magnification. These can be observed in Fig. 1.

Table 1 Histologic criteria for assessment of pulp reactions

Histologic assessment	Histologic criteria
Pulp inflammation	
None	The pulp contained few inflammatory cells, or an absence of inflammatory cells.
Slight	The pulp had localized inflammatory cell lesions predominated by polymorphonuclear leucocytes, or mononuclear mononuclear lymphocytes associated with the site of pulp exposure.
Moderate	The pulp had polymorphonuclear leucocyte lesions involving more than one-third of the coronal pulp.
Severe	Following chronic inflammatory cell activity, the pulp tissue is largely necrotic.
Dentine bridge	The area of tertiary dentine secreted by odontoblast-like cells at the site of pulp exposure.
Odontoblast cells	Primary palisade columnar cells with an eosinophilic cytoplasm and a nucleus located in a basal position, adjacent to the predentine.
Odontoblast-like cells	Newly differentiated cells with a similar phenotype to odontoblast cells, these cells have a tubular continuity with dentine bridge formation.
Reorganizing tissue	Newly differentiated fibroblast cells with a mixed morphology associated with odontoblast-like cells.
Operative debris	Dentine chips, particles of restorative material and globules of adhesive observed after direct pulp capping.
Tunnel defect	A discontinuity of complete dentine bridge formation across the site of pulp exposure, sometimes containing cells and called tissue tracts.

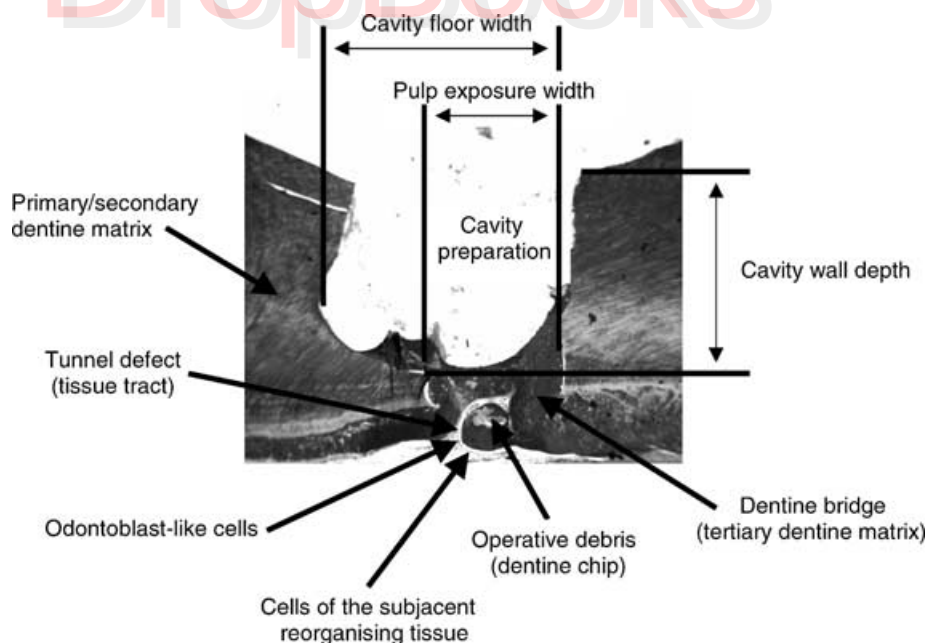


Figure 1 Histology of the pulp-exposed cavity preparation.

Histomorphometry

Hundreds of histological sections were cut from the same tooth, each of the sections was examined and a representative section from each tooth with typical histology was selected for histomorphometric analysis. The area of tertiary dentine bridge in representative sections was measured histomorphometrically at $\times 100$ magnification using a grid eyepiece graticule, by counting the number of micrometer squares occupied by the bridge. The graticule was also used to measure the width of the pulp exposure, cavity floor width and cavity wall depth (Fig. 1) (Murray *et al.* 2000). The volume of the pulp-capping material was estimated using the formula, $V = \pi r^2 h$. Odontoblast cells were identified as primary palisade columnar cells with an eosinophilic cytoplasm and a nucleus located in a basal position adjacent to the predentine (Table 1). Odontoblast-like cells were identified using previously published criteria (Mjör *et al.* 1991, Tziafas 1994, Murray *et al.* 2002). These newly differentiated cells have a similar phenotype to odontoblast cells, but with a tubular continuity with dentine bridge secretion (Table 1). The density of the cells of the subjacent organizing tissue (Cox & Bergenholtz 1986, Mjör *et al.* 1991) was also measured in the same teeth (Table 1). This tissue equates with the subodontoblast layer observed beneath odontoblasts during primary dentinogenesis. Cell density was measured at the site of pulp exposure along the dentine bridge border per 0.0199 mm^2 unit area. The cells and dentine bridge border can be seen in Fig. 2. To remove the possible influences of mismatches within the data, each of the restoration variables between the CR, RMGI, $\text{Ca}(\text{OH})_2$

pulp capping groups were measured and compared with each other. All data were analysed statistically using analysis of variance (ANOVA) tests at a confidence level of 95% (STAT view software, SAS Inc., Cary, NC, USA).

Results

Odontoblast-like and subodontoblast cells

Odontoblast-like cells had a distinctive elongated columnar morphology, with a clear nuclear, (densely staining) cytoplasmic and secretory polarity, resembling that of original primary odontoblast cells. These cells were intimately positioned at the dentine bridge border, in a polarized alignment with the direction of dentine bridge formation (Fig. 2). The cells of the subjacent reorganizing tissue comprised of a mixed range of cells with differing morphologies, including fibroblasts and inflammatory cells (Fig. 2). These cells were distinguished from odontoblast-like cells for the following reasons. Cells within the subjacent reorganizing tissue were generally much smaller, and contained less cytoplasm, giving the appearance of nonsecretory cells. These cells did not appear columnar, polarized or in contact with the dentine bridge. The reorganizing tissue contained more cells per unit area and few fibroblasts, whereas the underlying pulp core contained a relatively sparse cell density, mainly comprising of fibroblasts.

Dentine bridge formation

The density of odontoblast-like cells was found to be the most important factor influencing dentine bridge

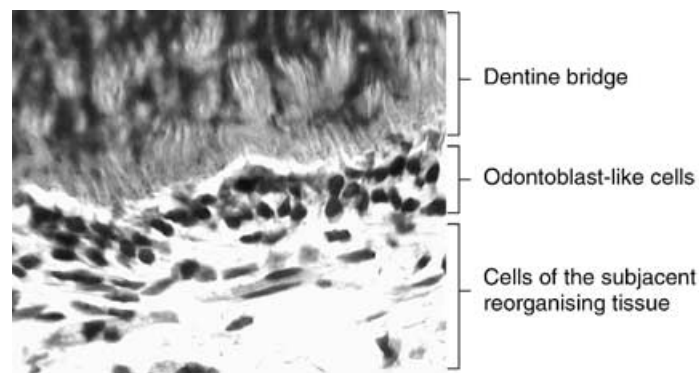


Figure 2 Histology of the odontoblast-like cells and the cells of the subjacent reorganizing tissue.

Following pulp exposure, dentine bridges are secreted by newly differentiated odontoblast-like cells. Cells of the subjacent reorganising tissue replace injured pulp cell populations.

Table 2 Hierarchy of pulp-capping variables correlated to odontoblast-like cell density

Pulp-capping variable	ANOVA <i>P</i> -values
Dentine bridge area	0.0001
Subjacent reorganizing tissue cell density	0.0001
Time elapsed since pulp capping	0.0014
Bacterial microleakage	0.0583
Operative dentine debris in bridge	0.1133
Volume of pulp capping material	0.3497
Category of pulp inflammation	0.4204
Cavity floor width	0.4807
Tunnel defects in dentine bridge	0.5236
Cavity wall depth	0.6293
Width of pulp exposure	0.7676
Operative dentine debris in pulp	0.8135
Pulp capping materials	0.8237

formation ($P = 0.0001$) (Table 2). Small densities of odontoblast-like cells were associated with less dentine bridge formation (Fig. 3). Bacterial microleakage appeared to have limited effect on the density of odontoblast-like cells ($P = 0.0583$) (Table 2; Fig. 3). Contamination of pulp exposures by bacterial microleakage reduced the dentine bridge secretory activity of odontoblast-like cells, especially when direct pulp capped with CR and RMGI, in comparison with $\text{Ca}(\text{OH})_2$ (Fig. 4). The presence of operative debris in dentine bridges increased the area of the bridge (Fig. 5), but had little effect on the density of odontoblast-like cells (Table 2). The area of dentine bridge formation was highly variable, and the width of pulp exposure appeared to have little effect on the area of dentine bridge formation (Table 2; Fig. 6).

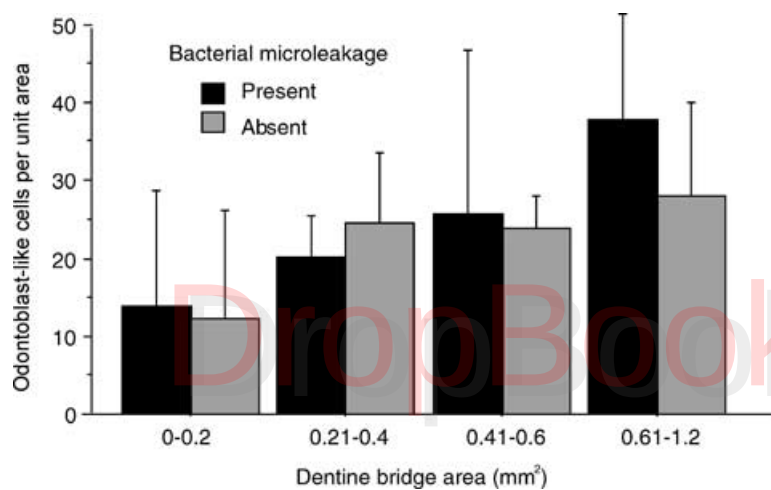
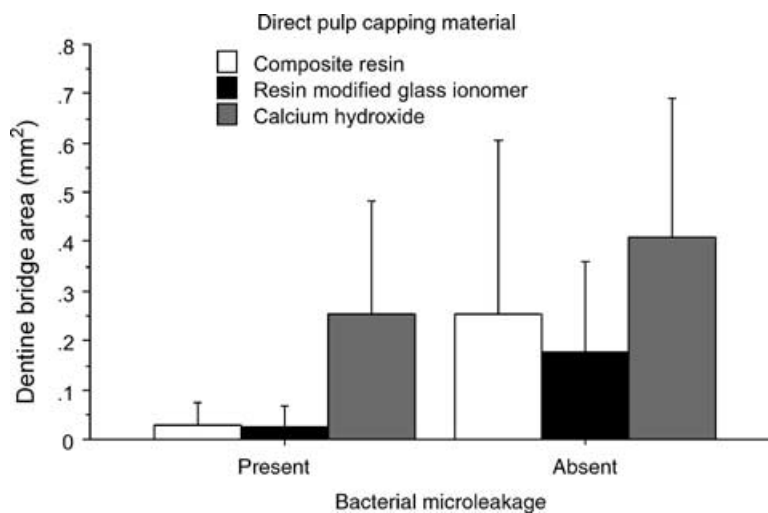
**Figure 3** The effect of odontoblast-like numbers and bacterial microleakage on the area of dentine bridge formation.**Figure 4** The effect of direct pulp-capping materials and bacterial microleakage on the area of dentine bridge formation.

Figure 5 The effect of direct pulp-capping materials and operative debris on the area of dentine bridge formation.

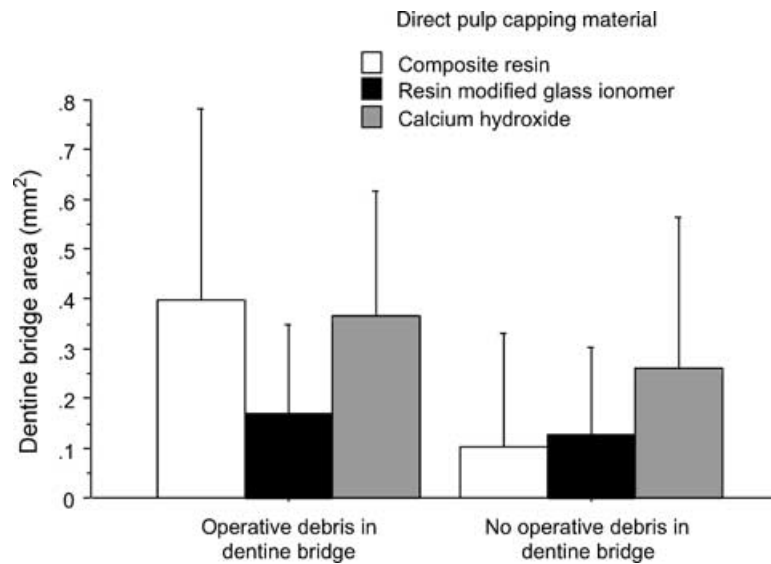
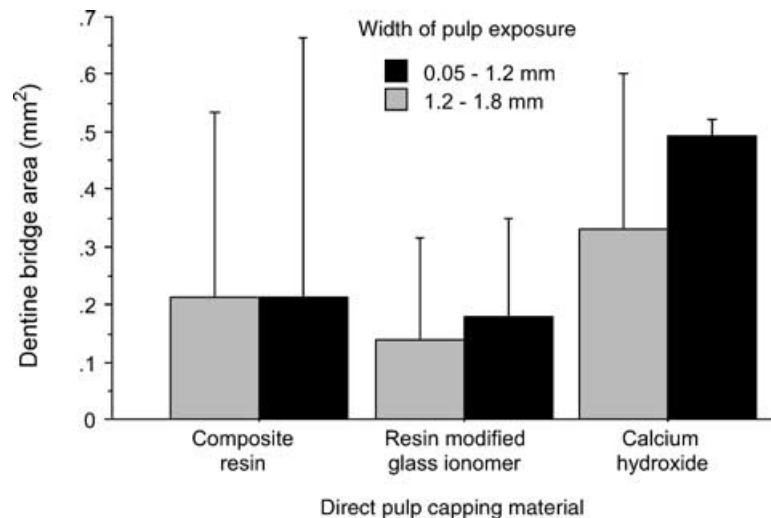


Figure 6 The effect of the width of pulp exposure and direct pulp capping materials on the area of dentine bridge formation.



Odontoblast-like cell density and reorganizing tissue cell density

The density of odontoblast-like cells was found to be correlated to the numbers of cells within the subjacent reorganizing tissue ($P = 0.0001$) (Table 2). Few odontoblast-like cells were observed in the absence of reorganizing tissue cells beneath pulp exposures (Fig. 7). Higher densities of reorganizing tissue cells were found to be associated with higher densities of dentine bridge secreting odontoblast-like cells (Fig. 7). The density of odontoblasts independent of the pulp exposure was also observed to have some association with the subjacent cell density

(Fig. 7). The ratio of odontoblast-like cell density to the density of the cells within the subjacent reorganizing tissue was maintained at a ratio of 0.553 (95% confidence interval 0.507–0.599 cells). Consequently, for every odontoblast-like cell, there were approximately two supporting cells in the subjacent reorganizing tissue.

Odontoblast-like cell density and direct pulp-capping materials

The selection of pulp-capping materials appeared to have little effect on the numbers of odontoblast-like cells beneath the site of pulp exposure (Fig. 8; Table 2).

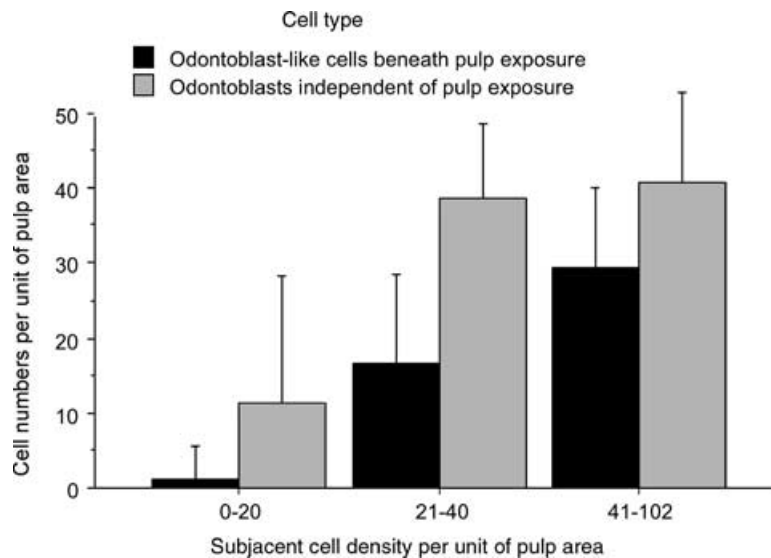


Figure 7 The effect of subjacent cell density on odontoblast-like and odontoblast cell density.

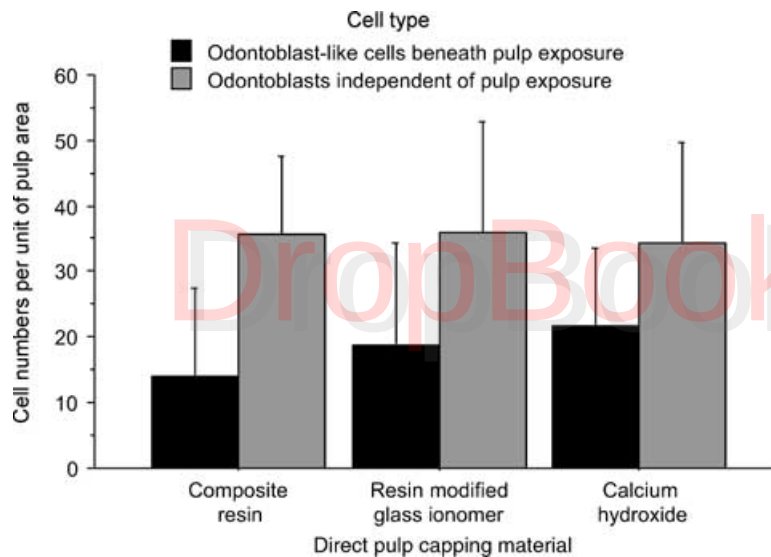


Figure 8 The effect of direct pulp-capping materials on odontoblast-like and odontoblast cell density.

However, the difference in cell density between the primary odontoblast cells, independent of the pulp exposure and new odontoblast-like cells at the site of exposure, was found to be over 50% (Fig. 8).

Odontoblast-like cell density over time

No odontoblast-like cells were observed at the site of pulp exposure at 7 days following pulp capping (Fig. 9). Meanwhile, odontoblast density was maintained independent of the site of pulp exposure ($P = 0.9424$)

(Fig. 9). The density of new odontoblast-like cells at the site of pulp exposure was observed to be a time dependent activity ($P = 0.0014$) (Table 2). The appearance of functional odontoblast-like cells were first observed at the 27-day examination point following the pulp exposure injury (Fig. 9). However, the density of odontoblast-like cells appeared to be only 69% of the odontoblast-like cell density observed at 97 days (Fig. 3). From 97 days up to 2 years following pulp capping, the density of odontoblast-like cells appeared to be maintained at a constant level (Fig. 9).

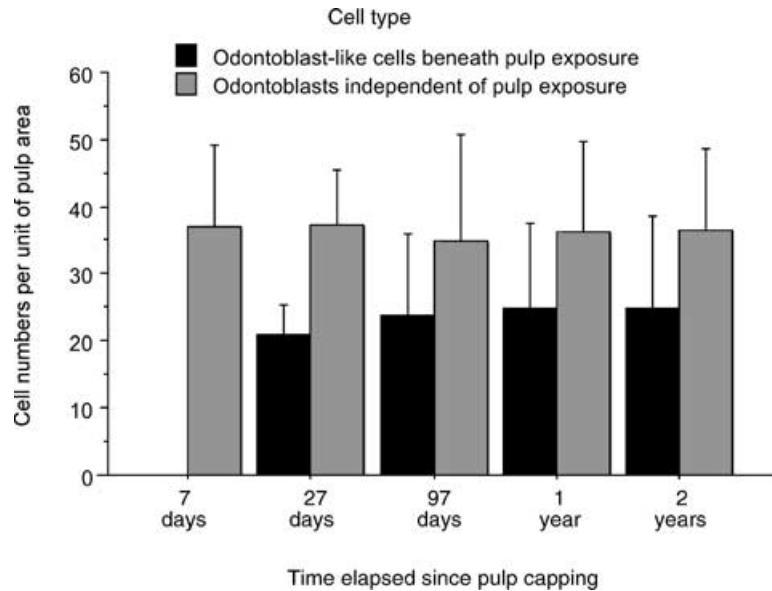


Figure 9 The effect of time on odontoblast-like and odontoblast cell density.

Odontoblast-like cell density and pulp inflammation

The category of pulp inflammation appeared to have little effect on the numbers of dentine bridge secreting odontoblast-like cells following pulp exposure (Table 2). Most of the teeth analysed had none, slight or moderate categories of inflammation and very few differences in cell numbers were observed (Fig. 10). However, in the presence of severe pulp inflammation, the mean numbers of both odontoblast-like (–76%) and odontoblast cells (–83%) were much reduced in comparison with the other categories (moderate) of pulp inflammation (Fig. 10).

Variables not correlated to odontoblast-like cell density

The following variables did not appear to be closely correlated to odontoblast-like cell density: dimensions of the cavity preparations and pulp exposure, tunnel defects in dentine bridge, operative dentine debris in bridge and pulp, and pulp capping material (Table 2).

Comparison of restoration variables

The guidelines of the International Standards Organization (ISO 7405) require established capping materials

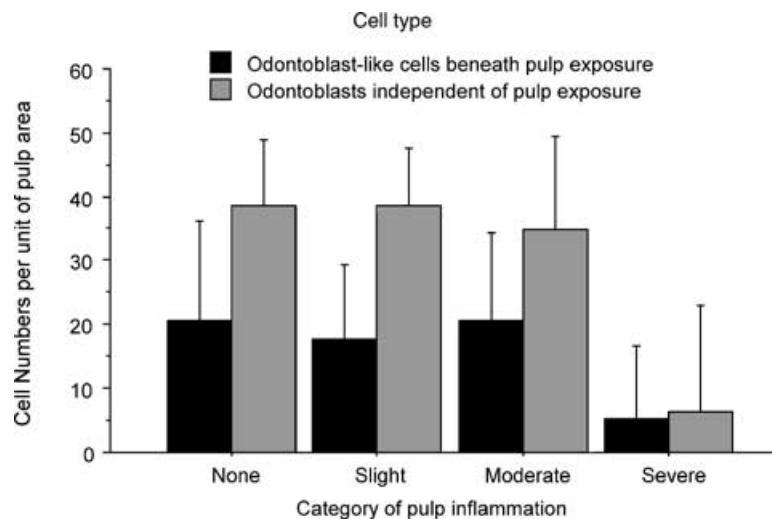


Figure 10 The effect of pulp inflammation on odontoblast-like and odontoblast cell density.

be used as control groups for measuring pulp responses, commonly $\text{Ca}(\text{OH})_2$ is used as the control group (Schröder 1985, Cox & Bergenholtz 1986, D'Souza *et al.* 1995) because it is generally the most well established material. No statistically significant differences were found between the use of $\text{Ca}(\text{OH})_2$ and RMGI and CR pulp-capping materials and pulpal activity. After comparing the data for differences between CR, RMGI, and $\text{Ca}(\text{OH})_2$ pulp-capping groups, no statistical differences were found at $P = 0.05$ significance level for time elapsed following surgery, the width of the pulp exposure, the volume of pulp-capping material or width of the cavity floor, thus ensuring that all the variables were evenly distributed.

Discussion

Treatment modalities for the exposed dental pulp have undergone numerous changes, recognizing that it is not easy to promote healing following pulp exposure (Schröder 1972, 1985). There have been several autoradiographic studies, which have examined the pulpal cell populations for their possible odontoblast-like progenitor potential (Fitzgerald *et al.* 1990), in addition to many experimental pulp-capping studies, which have shown the importance of the prevention of bacterial microleakage by pulp-capping materials (Cox *et al.* 1987). This present study investigated noninfected mechanical pulpal exposures; the only source of infection was by bacterial microleakage through the cavity margins. In situations of accidental trauma and caries pulp exposure, it is likely that the pulp tissue will be infected with bacteria prior to pulp capping, and this will probably cause more severe degenerative reactions and impaired reparative responses. In the controlled conditions of this present study, it was not possible to mimic caries or accidental pulp exposure. However, it was possible to correlate the effects of the variables of pulp-capping treatments directly to the kinetics of odontoblast-like cell formation and their dentine bridge secretory activity following mechanical pulp exposure. This study attempted to scientifically evaluate pulp capping and quantify the effects and importance of pulp-capping variables in relation to odontoblast-like cell activity. This investigation is different from previous reports of pulp capping and dentine bridge formation because of the focus on pulp tissue regeneration, especially the odontoblast-like cells responsible for secreting dentine bridges. This shift in emphasis is necessary because the application of tissue engineering to accomplish restorative treatment requires an understanding of how pulp tissue can be regenerated, and this information is scarce.

Calcium hydroxide is the most commonly used pulp-capping material, and it has been claimed to promote dentine bridge formation (Schröder 1972, 1985). However, $\text{Ca}(\text{OH})_2$ did not appear to influence odontoblast-like density in comparison with CR or RMGI pulp capping. This finding is in agreement with studies that have demonstrated the natural ability of the pulp to form dentine bridges under most types of pulp-capping materials, as long as a bacteriometric seal is provided (Cox *et al.* 1987). Also, in agreement with these previous studies, it was observed that bacterial microleakage reduced the odontoblast-like secretion of dentine bridges. Although there are many properties that are necessary for dental materials to function adequately, from our findings, it is postulated that one of the most important properties of a pulp-capping material is its capacity to prevent bacterial microleakage. Bacterial microleakage can impede bridge formation; this may lead to the development of postoperative complications if the dentine bridge cannot protect the pulp from injury. Odontoblast-like cell density did not appear to be readily influenced by the effects of pulp-capping variables, which suggests that complications with dentine bridges result from the inhibition of odontoblast-like dentine bridge secretory activity, rather than the inhibition of odontoblast-like cell density. All of these variables require further investigation to establish the precise nature of their effects; however, it is apparent that the outcome of pulp-capping treatments could be beneficially influenced by directing attention to treatment factors which prevent bacterial microleakage.

Whilst it has been established that odontoblast-like cells require adhesion to an appropriate surface before cell differentiation and dentine bridge formation can begin (Veis 1985), little attention has been directed towards the possible role of the cells of the subjacent reorganizing tissue. Our observations show that the density of cells within the reorganizing tissue is closely associated with the odontoblast-like cells following pulp capping. The role of these cells remains unclear, although they may well have a supporting function in relation to odontoblast-like cell activity. Approximately two cells per unit area of the subjacent reorganizing tissue, appear to be necessary to support odontoblast-like cell density per unit area beneath dentine bridge formation. Previous investigations have observed 'cell-rich reorganizing tissue' subjacent to odontoblast-like cells (Cox & Bergenholtz 1986, Mjör *et al.* 1991), and the importance of the organizing tissue to the differentiation and function of odontoblast-like cells is now clear.

The present study has not directly addressed the mechanisms by which odontoblast-like cell differentiation and reparative dentinogenesis take place leading to dentine bridge formation. However, this study has highlighted the exquisite regenerative potential of the dental pulp. The beginning of odontoblast-like cell differentiation and secretory activity between 7 and 27 days is in agreement with observations of the beginning of dentine bridge formation in human pulp-capped teeth (Schröder 1972). Similar time scales were observed in the ferret after capping with isolated dentine matrix components (Smith *et al.* 1990), and growth factors, particularly of the TGF- β family, have been implicated in the signalling of odontoblast-like cell differentiation. Implantation of TGF- β 1 has been shown to induce odontoblast-like cell differentiation in pulp tissue (Tziafas 1994), and release of endogenous pools of this growth factor from the matrix of the dentine pulp may mediate cellular signalling in clinical situations of pulp capping.

Unfortunately, it was not possible to characterize the migration and differentiation of pericytes from endothelial cell walls. Their fibroblast phenotype and the lack of available markers require a reliance on location and activity to identify these cells. Unfortunately, pericytes have no other specific characteristic (Carlile *et al.* 2000). Only when such cells had differentiated into an odontoblast morphology (Mjör *et al.* 1991, Tziafas 1994, Murray *et al.* 2002) was it possible to characterize the activity of these cells. Odontoblast-like cells differentiate at a rapid rate following exposure, but their rate of differentiation slows and appears completed within 100 days of exposure. Cells within the subjacent reorganizing tissue are required to permit odontoblast-like cells to differentiate and become functionally active. The number of functional odontoblast-like cells mediates the rate of dentine bridge formation and the time elapsed since pulp exposure. Bacterial microleakage can impede the secretion of dentine bridge by odontoblast-like cells. Odontoblast-like cells appear conserved beneath dentine bridges for at least 2 years following pulp exposure, suggesting they remain permanently to provide pulp protection. These observations provide new insight into the kinetics of odontoblast-like activity during tooth repair activity. The challenge for the future is to manage the activity of the odontoblast-like cells in order to accomplish successful pulp capping therapy in cases where the prognosis is conventionally deemed to be poor. In the meantime, concentrating attention on limiting the width of pulp exposure may beneficially influence pulp-capping therapy; this is because the availability of odontoblast-like cells appears to be strictly limited in

number. In the present study, dentine bridging was observed following pulp exposures of up to 1.2 mm in width. But, the secretion of dentine bridges over greater widths of pulp exposure may be more problematic. This is because there appears to be a very limited number of odontoblast-like cells able to secrete dentine bridges. In the absence of odontoblast-like cells able to align along the complete site of pulp exposure, incomplete bridging, tunnel defects and tissue tracts are likely to appear where no dentine bridge secretion has taken place. These defects in the continuity of dentine bridge formation will allow the penetration of bacterial microleakage into the pulp tissue if the sealing of the direct capping material at the cavity margin should fail. Larger sizes of pulp exposure are also likely to generate a greater quantity of operative debris that will impair the continuity of dentine bridging and complicate pulp tissue healing. Operative debris appears to increase the area of dentine bridge; large dentine bridges are not beneficial because these will make future endodontic treatment difficult or impossible. Most important is the placing of capping materials that prevent bacterial microleakage over the long term. This is because bacteria impede dentine bridge formation, which will reduce pulp healing and protection. In addition, bacteria will stimulate pulp inflammation, creating healing complications that could lead to treatment failure.

References

- About I, Bottero M-J, de Denato P, Camps J, Franquin J-C, Mitsiadis TA (2000) Human dentin production *in vitro*. *Experimental Cell Research* **258**, 33–41.
- Alliot-Licht B, Hurtrel D, Gregoire M (2001) Characterization of smooth muscle actin positive cells in mineralized human dental pulp cultures. *Archives of Oral Biology* **46**, 221–8.
- Carlile MJ, Sturrock MG, Chisholm DM, Ogden GR, Schor AM (2000) The presence of pericytes and transitional cells in the vasculature of the human dental pulp: an ultrastructural study. *Histochemical Journal* **32**, 239–45.
- Cox CF, Bergenholtz G (1986) Healing sequence in capped inflamed pulps of Rhesus monkeys (*Macaca mulatta*). *International Endodontic Journal* **19**, 113–20.
- Cox CF, Keall CL, Keall HL, Ostro E, Bergenholtz G (1987) Biocompatibility of surface-sealed dental materials against exposed dental pulps. *Journal of Prosthetic Dentistry* **57**, 1–8.
- Cox CF, Sübay RK, Ostro E, Suzuki S (1996) Tunnel defects in dentin bridges: their formation following direct pulp capping. *Operative Dentistry* **21**, 4–11.
- D'Souza RN, Bachman T, Baumgardner KR, Butler WT, Litz M (1995) Characterization of cellular responses involved in reparative dentinogenesis in rat molars. *Journal of Dental Research* **74**, 702–9.

- Feit J, Metelova M, Sindelka Z (1970) Incorporation of 3H-thymidine into damaged pulp. *Journal of Dental Research* **49**, 783–5.
- Fitzgerald M, Chiego DJ Jr, Heys DR (1990) Autoradiographic analysis of odontoblast replacement following pulp exposure in primate teeth. *Archives of Oral Biology* **35**, 707–15.
- Hanks CT, Fang D, Sun Z, Edwards CA, Butler WT (1998) Dentine-specific proteins in the MDPC-23 cell line. *European Journal of Oral Sciences* **106** (Suppl. 1), S260–S6.
- ISO Technical Report 7405 (1980) International Organization for Standardization. Recommended standard practices for biological evaluation of dental materials. *International Dental Journal* **30**, 140.
- McKay GS (1970) Gram stain modified to improve colour contrast. *Journal of Clinical Pathology* **23**, 191.
- Mjör IA (1983) Biological and clinical properties. In: Mjör IA, ed. *Dental Materials, Biological Properties and Clinical Evaluation*. Boca Raton: CRC Press, 91–121.
- Mjör IA, Dahl E, Cox CF (1991) Healing of pulp exposures: an ultrastructural study. *Journal of Oral Pathology* **20**, 496–501.
- Murray PE, About I, Lumley PJ, Smith G, Franquin J-C, Smith AJ (2000) Postoperative pulpal and repair responses. *Journal of the American Dental Association* **131**, 321–9.
- Murray PE, Hafez AA, Smith AJ, Cox CF (2002) Hierarchy of pulp capping and repair activities responsible for dentin bridge formation. *American Journal of Dentistry* **15**, 236–43.
- Schröder U (1972) Evaluation of healing following experimental pulpotomy of intact human teeth and capping with calcium hydroxide. *Odontologisk Revy* **23**, 329–40.
- Schröder U (1985) Effects of calcium hydroxide-containing pulp-capping agents on pulp cell migration, proliferation, and differentiation. *Journal of Dental Research* **64**, 541–8.
- Smith AJ, Tobias RS, Plant CG, Browne RM, Lesot H, Ruch JV (1990) *In vivo* morphogenic activity of dentine matrix protein. *Journal de Biologie Buccale* **18**, 123–9.
- Tarim B, Hafez AA, Cox CF (1998) Pulpal response to resin-modified glass-ionomer material on nonexposed and exposed monkey pulps. *Quintessence International* **29**, 535–42.
- Tziafas D (1994) Mechanisms controlling secondary initiation of dentinogenesis: a review. *International Endodontic Journal* **27**, 61–74.
- Veis A (1985) The role of the dental pulp – thoughts on the session of pulp repair responses. *Journal of Dental Research* **64**, 552–4.

DropBooks